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SPIROCHAETA SUIS, ITS SIGNIFICANCE AS A PATHOGENIC ORGANISM.*

STUDIES ON HOG CHOLERA.

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(WITH PLATE 4.)

INTRODUCTION.

Since results of the dark field study of the blood of animals suffering from hog cholera were reported,[1] the work has been continued and several new phases of the problem have been investigated. In the former article it was reported that a relatively large spirochete and characteristic granules were present in the blood of 40 hogs infected with cholera, representing 12 different strains of virus, while the blood of 48 normal hogs was observed to be free from spirochetes and granules. The spirochetes observed in specimens of blood from cholera hogs were very few in number, but the characteristic granules were apparently always present during the fastigium and were regarded as important findings.

In conjunction with the later studies of this spirochete in the blood, many observations were made by means of the dark field of material taken from the intestinal ulcers of hogs dead from hog cholera. Dark field examinations have been made of the necrotic tissue from the intestinal ulcers of all animals dead from hog cholera during the course of our experiments of the last six months. Scrapings from the intestinal mucosa and contents of the crypts in the ceca of normal hogs, some of these being susceptible to hog cholera, others immune to the disease, were also examined at every opportunity. In all, the study with the dark field of material from the crypts and ulcers of normal and diseased hogs has included about 140 cases. As a result of this work, the details of which will be published separately, we are inclined to believe that the following observations are true.

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* Received for publication August 11, 1913.

[1] King, Baeslack, and Hoffmann, *Jour. Infect. Dis.*, 1913, 12, p. 206.

1. The mucosa of the large intestine, particularly that of the cecum, of normal hogs, includes in its flora non-pathogenic spirilla and spirochetes, relatively large forms predominating.

2. In the ulcerated patches of cecal mucosa and in the crypts, near the ileo-cecal valve, of hogs dead from cholera is localized a constant species of spirochete, which is pathogenic for swine and which plays an important part in the production of hog cholera.

3. The crypts and healed ulcers of hogs actively immunized against hog cholera may contain, for a variable period of time after immunization, in addition to non-pathogenic spirochetes, the same species of pathogenic spirochete referred to above.

There are many factors entering into the above tentative conclusions which cannot be discussed in detail in this paper, but which will follow in future reports of our studies on this disease. Among the more important observations, however, are the results obtained from the experimental curative treatment of cases of hog cholera with arsenical and mercurial preparations. This work resulted, not in cures, but, usually, in prolongation of life, which, in most cases, was coincident with an apparent decrease in number or total disappearance of the spirochetes in question from the necrotic tissue in the cecal ulcers. Positive dark field findings of spirochetes in intestinal ulcers of cholera hogs are in accordance with the results reported by von Rüther,[1] Uhlenhuth and Haendel,[2] and Betegh.[3] The presence of spirochetes in the bile of cholera hogs, as reported by Uhlenhuth and Haendel, has not been found in the cases examined thus far.

On account of the fact that efforts to secure cultures or satisfactory stained preparations of *Spirochaeta suis* from the blood of cholera hogs were not successful, and because of the large amount of bacterial contamination present in the intestinal lesions, attempts were made to find localized foci of the organism elsewhere in the diseased body. Microscopical preparations and cultures were made from the spleen, liver, lymphatic glands, spinal cord, cerebrospinal fluid, bile, and urine. These attempts were unsuccessful except in one instance. In one case (Hog 615, inoculated subcutaneously with exudate from local lesion, Hog 599), the dark field revealed in the inguinal lymph gland a spirochete resembling the assumed pathogenic form. In many of the dark field preparations numerous granules were observed, but it was impossible to draw any definite conclusions as to their origin and relationship to *Spirochaeta suis* or to tissue elements.

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[1] *Berl. tierarztl. Wchnschr.*, 1911, 27, p. 191.

[2] *Handbuch d. path. Mikroorg.*, 1913, 6, p. 337.

[3] *Berl. tierarztl. Wchnschr.*, 1912, 28, p. 968.

Finally, when an opportunity presented itself, dark field examinations were made of external lesions on the leg and ear of a case of cholera of the chronic type (Hog 583, May 8, 1913) and they were found to contain spirochetes, presumably of one species, in large numbers. So far as we know, similar observations have never been reported except by Dodd[1] of Pretoria, Transvaal. In describing "a disease of the pig due to a spirochete," Dodd states that he found a spirochete in the cutaneous lesions of a number of pigs. The disease could not be transmitted experimentally by the inoculation of blood from an infected animal, but was transmitted, however, by actual contact. The infection was fatal to several pigs. Autopsy revealed nothing except the local skin lesions and general anemia. The disease appeared to be some cutaneous infection due to the spirochete found in the local lesions. Scrapings from the skin of healthy hogs and from the normal skin of the affected pigs were found to contain no spirochetes.

Since the first observation we have not only found that all external local lesions examined, including 11 cases of hog cholera, were constant foci for *Spirochaeta suis*, but we have also been able to produce typical hog cholera, with local external lesions, by inoculating a small bit of the tissue or a small amount of exudate from local lesions of the affected animal into a healthy pig. Scrapings from the healthy skin of hogs and from the areas of discoloration on the ventral surface of the body of animals suffering from cholera have been submitted to dark field examination with negative results. The results of this work, which is still in progress, will be published later.

In our former paper, the spirochete observed in the blood of hogs infected with hog cholera was described as follows:

"In the specimens of blood from all infected hogs, which have been observed by means of the dark field, a relatively large spirochete has been found. It averages

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[1] *Jour. Comp. Path. and Ther.*, 1906, 19, p. 216.

> from five to seven microns in length and one micron in width. The body of the organism is flexible and round at its ends. It presents no knobbed appearance at its poles Actively motile, it revolves about its longitudinal axis. Its motility is undulating in character and its spirals are fixed. A few of these organisms have been observed dividing longitudinally. In one permanent microscopical mount, prepared by india ink fixation, one of these organisms apparently shows a polar flagellum. On the dark field this spirochete is readily distinguished from bacteria on account of its lack of rigidity and its characteristic motility, and from 'blood filaments' by its greater refractive properties and characteristic morphology.
>
> "This spirochete has not been found in large numbers, in any of the blood preparations. However, in nearly every specimen examined, more than one has been observed and in many cases five or six have been found with little difficulty. As a rule the organisms have been found to be more numerous at the height of the disease. The specimens of blood examined have been diluted in the proportion of about one to ten or fifteen with sterile sodium citrate solution, which factor should be considered in contemplating the number observed in a given positive specimen. Moreover, it is suggested that this organism, when observed as a spirochete form, constitutes only one stage of its development."

This description appears to be correct, except that we have never verified the presence of a polar flagellum.

It must be conceded that any successful dark field investigation requires patient work and much practice in interpretation. Therefore, one unacquainted with the dark field should not expect to obtain immediate satisfactory results from the study of blood. An intimate course of study is necessary to enable one to differentiate between "filaments," artefacts, and organisms of the morphological nature of the spirochete described in the blood of cholera hogs.[1]

The last statement quoted we now accept as a conclusion rather than a suggestion. Results lead us to believe that the spirochete observed in few numbers in the blood of cholera hogs is a transitional form.

The spirochete, which is constantly observed in the ulcerated mucosa and crypts in the ceca and in the external local lesions of cholera-infected animals, is an actively motile, comparatively long, spiral-shaped organism, of slight thickness. Measurements show that it is approximately 6–10 microns in length, averaging about

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[1] In this connection we were especially fortunate in the collaboration of our colleague, Dr. F. W. Baeslack. Because of his experience with the dark field in his study of *Spirochaeta pallidum* (see *Jour. Infect. Dis.*, 1913, 12, p. 55) and in his experimental observations of certain Trypanosomes, our dark field study of hog cholera blood was placed to great advantage. It is a pleasure to acknowledge his valuable assistance in that work, as well as the many helpful suggestions he has offered in our present investigation.

7 or 8 microns, and 0.3–0.6 micron in width. The convolutions vary from three to ten in number, rarely more than eight, and the spirals are fixed. It has been observed to divide longitudinally, and morphological variations, such as described by Gleitsmann[1] as a part of the life cycle of the Sudanspirochaetae, have been observed.

In our former report on the dark field study of the blood, observations relative to the presence of granules were summarized as follows:

> "Certain types of granules appear to be characteristic of blood from cholera hogs. It usually contains many granules, some very fine yet more distinct than blood dust, some larger still, and some very distinct highly refractive bodies. In many specimens of cholera blood were observed innumerable small granules, which were much more definite and distinct in outline than blood dust, and easily differentiated from the whitish, partially refractive granules from ruptured leukocytes and from the more highly refractive and larger bodies composed of débris, bacteria, and filaments. In this work the presence of these granules appeared to be so characteristic of blood from cholera hogs that it became an invaluable aid in finding the spirochetes. While these particular bodies may be disintegrated blood elements resulting from disease processes, yet it may properly be suggested that some of them may represent certain stages in the life cycle of the spirochete which has been observed."

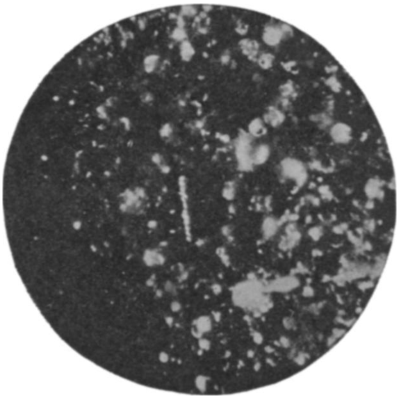

FIG. 1.—Photomicrograph of *Spirochaeta suis* in exudate, ear lesion, Hog 626. ×1200. (Dark field preparation.)

As the result of continued investigations, we believe that the granules represent an important factor in the life history of *Spirochaeta suis*. References to similar observations in the study of various spirochetes are found in the writings of Balfour,[2] Mühlens,[3] Ross,[4] McDonagh,[5] Dutton,[6] Mayer,[7] Mackinnon,[8] Schellack,[9] Bosanquet,[10] Marchoux and Couvy,[11] and others.

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[1] *Centralbl. f. Bakteriol.*, I, *Orig.*, 1913, 68, p. 31.

[2] *Report IV, Wellcome Research Lab.*, p. 76.

[3] *Handbuch d. Path. Protozoen, Prowazek*, Leipzig, 1912, p. 361.

[4] *Brit. Med. Jour.*, 1912, p. 1651.

[5] *Lancet, Proc. Roy. Soc.*, 1913, 6, p. 86.

[6] *Jour. Trop. Med.*, 1907, 10, p. 385.

[7] *Arch. f. Schiffs.- u. Tropenhyg.*, 1908, 12, Beihefte 1, p. 1.

[8] *Parasitology*, 1909, 2, p. 267.

[9] *Arb. a. d. k. Gsndhtsamte*, 1909, 30, p. 351.

[10] *Spirochaetes*, Saunders, 1911.

[11] *Ann. de l'Inst Past.*, 1913, 27, p. 450.

In cultures made from material containing *Spirochaeta suis* the presence of small granular forms is quite constant. As these appear in dark field preparations from cultures, they resemble those which were observed in the blood, and are seen in the exudate from local lesions, of cholera-infected hogs. They are relatively highly refractive, smaller than cocci, peculiarly distinct in appearance from foreign material, and possess more than the characteristic Brownian movement. The assumption that these granules form an integral part of the biologic processes of the organism, *Spirochaeta suis*, is substantiated by the results of the detailed experiments which follow.

Recognizing the difficulties involved in attempting to study *Spirochaeta suis*, and in determining its possible significance as a pathogenic form, our efforts have been directed toward the cultivation of the organism. This work has included the inoculation of artificial culture media with blood, intestinal ulcers, urine, contents of local external lesions, and other tissues from cases of hog cholera, followed by careful dark field examinations.

CULTIVATION OF SPIROCHAETA SUIS.[1]

A great many plantings on a variety of artificial culture media have been made from the blood, intestinal ulcers, and external local lesions of cholera-infected animals. These cultures have been made chiefly in deep horse serum media, to which have been added various chemicals in different proportions. All have been grown under strict anaerobic conditions in special electric incubators. Various means were used in attempting to eliminate contaminating organisms, which were always present in cultures made from the ulcers and, to a lesser extent, in those made from material in local lesions.

The culture medium which proved most successful consisted of a mixture of horse serum and pyrogallic acid, in which was placed fresh rabbit kidney at the time of inoculation. The pyrogallic medium of Proca, Danila, and Stroe[1] was made up in lots of one liter each by adding to 900 c.c. of horse serum 100 c.c. of pyrogallic acid mixture. The pyrogallic solution should be somewhat aged. A stock solution was therefore kept, which consisted of pyrogallic acid, 10 gm.; sodium hydroxid, 20 gm., and water, 1,000 c.c. After the addition of this mixture to horse serum and distribution in tubes the medium was heated one hour at 60° C. on each of three consecutive days.

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[1] As the proof of this paper is being read we note an article in current literature by Pfeiler and Lentz (*Berl. tierärztl. Wchnschr.*, 1913, 29, p. 690), who present the case histories of 15 pigs which died after having received injections of cultures made from the Berkefeld filtrates of serum and tissue exudates from hog cholera infected animals. These cultures represented transfers in the first, second, third, and fourth generations. The authors carefully avoid any statements which might indicate the nature of the cultures. No mention is made of the character of the organism in their cultures, the composition of their culture medium, or how long or under what conditions the cultures were incubated. On account of the conspicuous lack of such important data one can form no conclusion as to their mode of procedure, and, hence, the results which they have published must be regarded with some skepticism.

While this pyrogallic serum medium does not eliminate all bacterial contamination, at the same time allowing the spirochete to grow, it was found to give better results than media containing formol, gentian violet, phenol, or other inhibitory substances. For uncontaminated material, such as Berkefeld filtrates of impure cultures of spirochetes, plain or ascitic horse serum media were utilized. Fresh rabbit kidney was added to all media used and care was exercised that the horse serum was only partially coagulated and soft in consistency when ready for use.

The cultures have been examined at different intervals of time, following inoculation and incubation, in order to detect any facts which might come to light concerning the life history, morphology, and physiological habits of the organism. The method of examining cultures consists simply in withdrawing, with a sterile capillary pipette, a small portion of culture from the lower layers of the medium and examining the same in microscopical mount by means of the dark field. The Giemsa method of staining is employed whenever permanent mounts are desired. After some practice one can readily detect, by the dark field, spirochetes and granules in fresh cultures in distinction from cocci, other bacterial contamination, crystals, and small particles of culture medium.

NOTES ON TRANSMISSION OF HOG CHOLERA BY MEANS OF CULTURES OF SPIROCHAETA SUIS.

I. ORIGINAL CULTURE.

Culture 551-A was secured from the ulcerated cecum of Hog 551. The account of this case may be found in the chart for Hog 551.[2]

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[1] *Compt. rend. Soc. de biol.*, 1912, 72, p. 895.

[2] Positive and negative signs indicate the presence or absence of *Spirochaeta suis* in the blood of the animal on dates indicated.

Cultures were made from the ulcerated cecum, spleen, and lymphatic glands of Hog 551. Those from the spleen and lymphatic glands developed no growth, while some of those from the cecum, after five days' incubation under anaerobic conditions, showed the growth of spirochetes which varied in form and size. Fig. 3, p. 212, in our former publication[2] shows a photomicrograph of a stained preparation made from this culture. When this culture was studied, our attention was directed especially to the larger forms of spirochetes which were found in it. This was due to the fact that we were familiar with the form which had been observed in the blood of cases of hog cholera. At this time no special attention was given to smaller forms representing more typical spirochetes which were present in this culture, as was shown, some weeks after the study of the culture, by careful re-examinations of the permanent stained preparations.

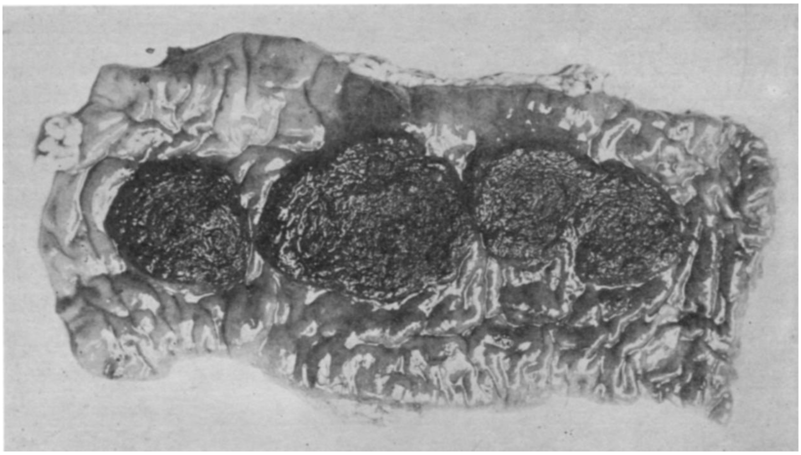

FIG. 2.—Ulcers in cecum of Hog 569.

Transfers were made from Culture 551-A and grown both aerobically and anaerobically. The species of bacteria which contaminated Culture 551-A were very carefully isolated and identified as follows: *B. pyocyaneus*, three strains; *B. coli communis*, five strains. These cultures, which contaminated 551-A, representing *B. coli communis* and *B. pyocyaneus*, were mixed and suspended in physiologic salt solution. Six cubic centimeters of this suspension on March 14 were injected intravenously into Hog 570. This animal showed no disposition to develop hog cholera, but did react to the injection of live *B. pyocyaneus* and *B. coli communis*, as the clinical chart for Hog 570 will show.

Culture 551-A was suspended in sterile physiologic salt solution and an intravenous injection of 10 c.c. given to Hog 559. The results of this inoculation are recorded in the chart for Hog 559.

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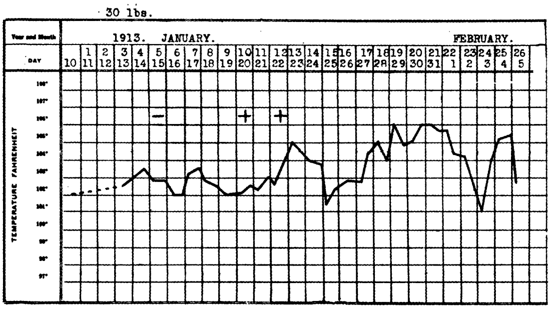
[2] *Jour. Infect. Dis.*, 1913, 12, p. 206.

HOG 551.



January 10. Received from Rochester, Mich.

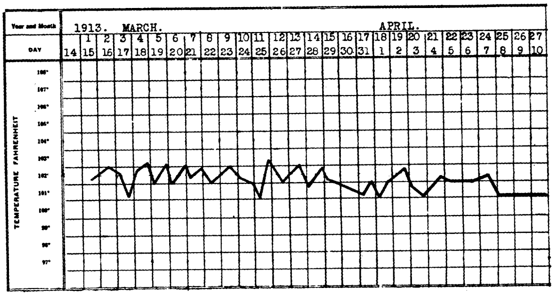
January 16. Intramuscular injection 8 c.c. of virus, Berkefeld filtered, 1:4 saline. Ohio (Pettigrew) strain.

January 31. Animal very weak.

February 5. Animal moribund, bled and examined.

Typical lesions of cholera: lungs, large areas of hepatization; kidney, petechiated, capsule non-adherent; lymphatic and inguinal glands, greatly enlarged and hemorrhagic; spleen, friable and engorged with blood, normal in size; cecum, large area of ulcers.

HOG 570.



March 14. Intravenous injection of 6 c.c. physiologic salt suspension cultures from cecum of Hog 559.

March 18–25. Slightly indisposed, appetite not good, otherwise condition practically normal.

March 31. Apparently in good condition. Released.

April 2. A little "off feed."

April 9. Fully recovered, released and put on other work April 14.

April 30. Animal found dead, result of intramuscular injection of 5 c.c. II Cycle virus. Typical lesions of hog cholera.

Hog 559 was in a perfectly healthy condition at the time of inoculation, February 10, and weighed approximately 75 pounds. Four days after inoculation, the temperature of the animal began to rise and on the fifth day symptoms of hog cholera appeared. The animal was slightly "off feed" and inclined to be inactive. Dark field examination of the blood at this time showed the presence of a few spirochetes and characteristic granules. After 48 hours the temperature dropped somewhat and for several days ranged between 103° and 104°. About 12 days after the inoculation the animal appeared to be brighter and seemed to be rapidly improving, but 15 days after inoculation a remission occurred, the temperature was elevated 2° during one afternoon, and from this time the animal gradually grew worse until death, which occurred 22 days after inoculation. The later symptoms consisted of weakness, inability to use hind quarters, bloody urine, mucous diarrhea, anorexia, and general malaise. On March 4 the animal was moribund and, after being photographed, was bled from the carotid artery and examined (see photograph of Hog 559). On autopsy typical lesions of hog cholera were found.

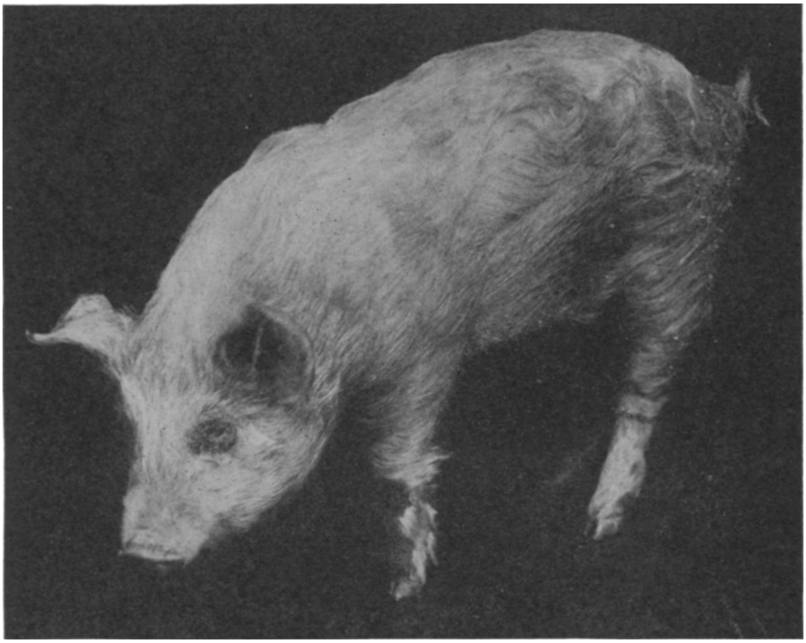
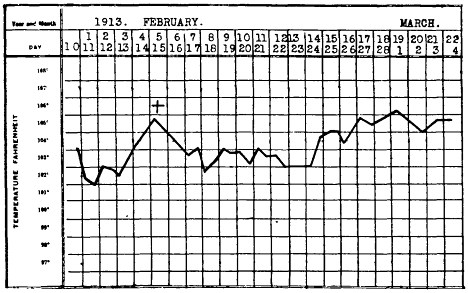


FIG. 3.—Hog 559.

Blood extravasations were present in the subcutaneous tissue, especially in the axillary region and lower abdominal region. Both the auricles and ventricles of the heart showed ecchymosis. The lungs contained numerous hemorrhagic infarcts and a few areas of hepatization. The liver appeared to be somewhat enlarged and contained areas of fatty degeneration. The spleen was slightly atrophied, tissue firm, but engorged with blood. Both kidneys were full of petechiae. The mucosa of the cecum and the large intestine was congested and marked with hemorrhagic spots throughout. Two typical button ulcers were found in the cecum. In the small intestine there were present two enlarged areas, consisting of necrotic intestinal wall surrounded by extensive hemorrhages. The peritoneum in the lower abdominal region showed hemorrhagic areas. The mesenteric, inguinal, and intraperitoneal lymph glands were enlarged and hemorrhagic (see Plate 4).

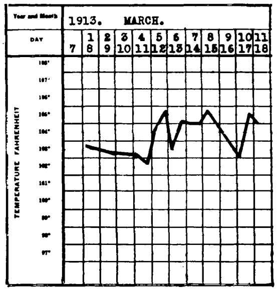
A specimen of blood serum from Hog 559 was filtered through Berkefeld, after being diluted 1:4 with physiologic salt solution, and 10 c.c. of the filtrate injected intramuscularly into Hog 569. This material proved to be virulent, as Hog 569 showed severe symptoms of hog cholera five days after inoculation and became moribund on the eleventh day. The autopsy showed classical lesions of hog cholera (see chart for Hog 569).

HOG 559.



February 10.	Intravenous injection 10 c.c. suspension physiological salt solution of Culture 551-A.
February 15.	Clinical symptoms present, appetite poor.
February 23.	Animal improved in condition.
February 25.	Remission.
March 4.	Animal moribund, and bled from carotid artery. Typical lesions: heart, lungs, kidney, cecum, and lymphatic glands.

HOG 569.



March 7.	Blood negative, free from granules.
March 7.	Intramuscular injection of 10 c.c. Virus 559, diluted 1:4, physiological salt and Berkefeld-filtered strain (Culture 551-A).
March 12.	Symptoms appeared.
March 18.	Animal moribund and bled from carotid artery. Lesions typical: heart, lungs, spleen, kidney, inguinal glands, and mesenteric lymph glands; ulcers in cecum.

With reference to infection from other material than that which was used in the inoculations, it should be stated that results from Hog 559, as well as all others used in this series of experiments, were carefully controlled by the presence of normal hogs in the experimental stable. The animals used in each series of experiments, as well as the normal animals, were kept in separate rooms. The normal animals were placed under exactly the same conditions and had the same care that was exercised in regard to the treated animals.

An additional control on this work depended upon the use of Hog 570, which was inoculated with the contamination present in Culture 551-A. Furthermore, it has been observed that the serum of Hog 559, filtered through Berkefeld, was capable of producing typical hog cholera in Hog 569.

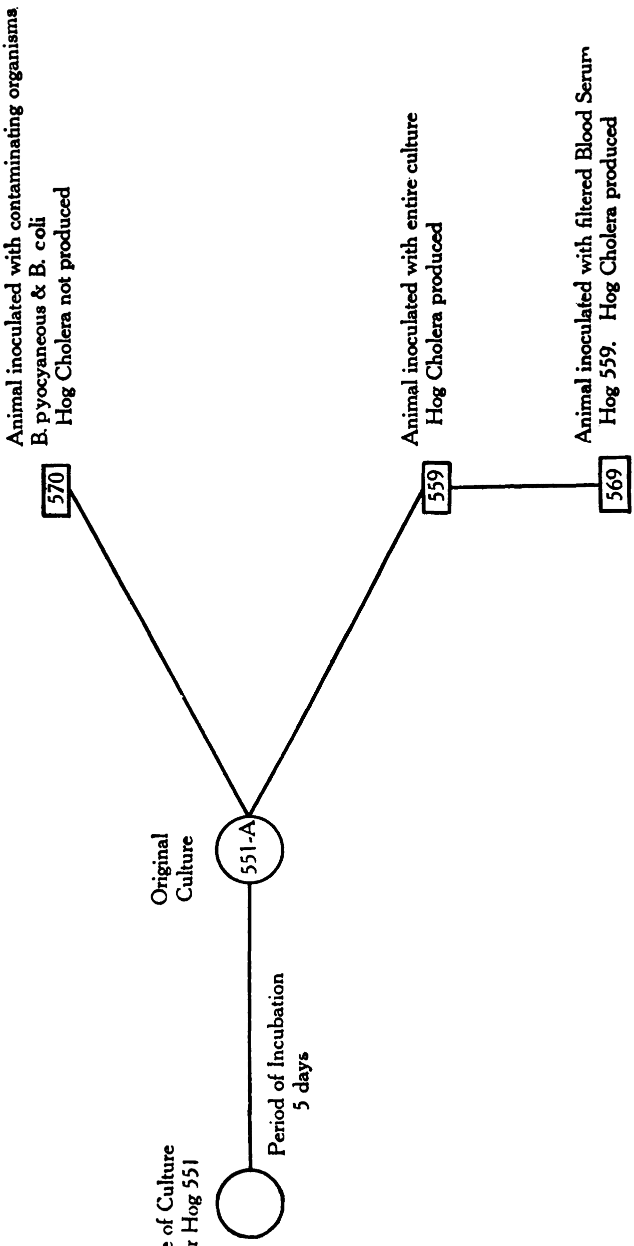

Source of Culture
Ulcer Hog 551
Period of Incubation
5 days
Original
Culture
551-A
570
Animal inoculated with contaminating organisms
B. pyocyaneous & B. coli
Hog Cholera not produced
559
Animal inoculated with entire culture
Hog Cholera produced
569
Animal inoculated with filtered Blood Serum
Hog 559. Hog Cholera produced

CHART 1.—Graphic representation of experiments with original culture from Hog 551.

Among the criticisms which may be offered on this experiment, the most important relates to the possibility of transferring the original virus from Hog 551 through Culture 551-A. This point could not be controlled, altho an estimate was made that Hog 559 received less than 1/750 c.c. of material originally obtained from the ulcer of Hog 551. It has been estimated by King and Wilson[1] that the minimum fatal dose of hog cholera virus, diluted in physiologic salt solution, is approximately 1/300 c.c. The above approximation that Hog 559 received about 1/750 c.c. was obtained on the basis of the utilization of about 1/15 gm. of ulcer from Hog 551 in approximately 10 c.c. of culture media. However, these estimates, including both the suggested minimum fatal dose of hog cholera virus in physiologic salt solution and the amount of original material transferred through Culture 551-A to Hog 559, are open to serious question in attempts to estimate whether or not any of the original virus from Hog 551 was transferred to Hog 559.

II. TRANSFER 1. SECOND GENERATION ON ARTIFICIAL MEDIA.

This culture was secured from the strain of virus from Hog 577. This animal received an intramuscular injection of 8 c.c. horse serum virus, diluted 1:4 in physiologic salt solution and Berkefeld filtered. The animal developed typical symptoms of hog cholera and after nine days, being moribund, was bled from the carotid artery and examined.

Hog 587 on April 11 received an intramuscular injection of 5 c.c. of serum from Hog 577. This animal promptly developed hog cholera of the acute type after five days' incubation and was bled and examined eight days after inoculation. The autopsy showed typical lesions of hog cholera of the acute type. Numerous blood extravasations were present in the subcutaneous tissue, especially in the axillary region. Both lungs were almost completely consolidated. The kidneys showed areas of cloudy swelling and petechiae near the upper ends. The spleen was engorged with blood. The mesenteric and inguinal glands were enlarged and hemorrhagic. The cecum contained a few small ulcers around the valve (see chart for Hog 587).

On April 19 cultures were made from the material obtained from the small ulcers in the cecum of Hog 587. These were incubated under anaerobic conditions for a period of 17 days and on May 5 dark field examination showed the presence of *Spirochaeta suis*. A variety of contaminating organisms was also present, both cocci and bacilli being represented in the contamination. The culture medium in the tube labeled "587-A" showed no liquefaction from the growth of the contaminating bacteria, and from it a number of transfers were made on May 5. On May 27, after incubation for 22 days, transfer labeled "Transfer 1-587-A," when submitted to dark field

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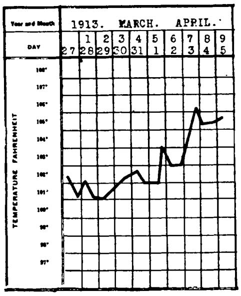
[1] *Bull. 171, Kansas State Agric. Coll.*, p. 163.

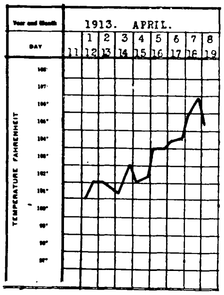
HOG 577.



March 27. Intramuscular injection 8 c.c. activated horse serum, diluted 1:4, physiological salt solution and Berkefeld filtered.
April 1. Symptoms appeared.
April 3. Animal very weak in hind quarters.
April 5. Animal moribund, bled and examined.

Typical lesions: heart, lungs, kidney, lymph glands, cecum; small intestine infected with *Ascariadae suella.*

HOG 587.

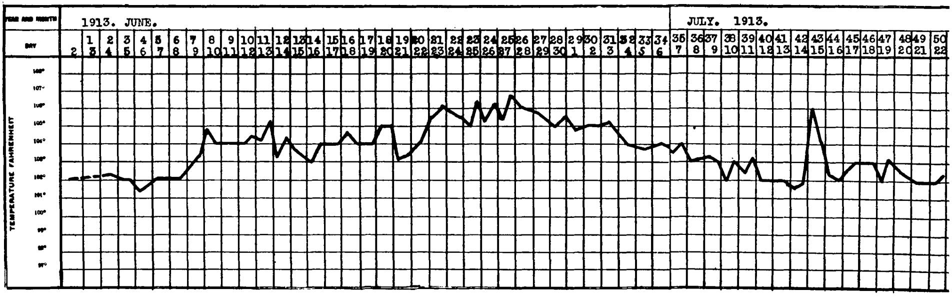


April 11. Intramuscular injection of 5 c.c. of Virus 577 I Cycle activated horse serum.
April 16. Symptoms of cholera appeared.
April 19. Animal moribund, bled from carotid artery and examined.

Lesions: lungs, spleen, cecum, subcutaneous tissue, and inguinal glands.

examination, showed a few *Spirochaetae suis* and numerous granules. All of the contaminating bacilli which were present in the original culture, 587-A, had disappeared and the only contamination present in the transfer was a micrococcus. A portion of this culture, Transfer 1-A-587-A, was broken up in sterile water and 1.5 c.c. of the suspension of this culture were inoculated, intramuscularly, into each of three hogs, Nos. 612, 613, and 614. Hogs 612 and 614, after eight days 'incubation, developed symptoms of hog cholera. After a few days the disease assumed the chronic form.

HOG 612.



June 2. Intramuscular injection 1.5 c.c. suspension Culture Transfer 1-587.
June 10. Symptoms appeared.
June 10–July 1. Chronic case of cholera.
July 4. Condition improved.
July 15. Animal had been fighting with strange hogs.
July 22. Released as healthy immune.

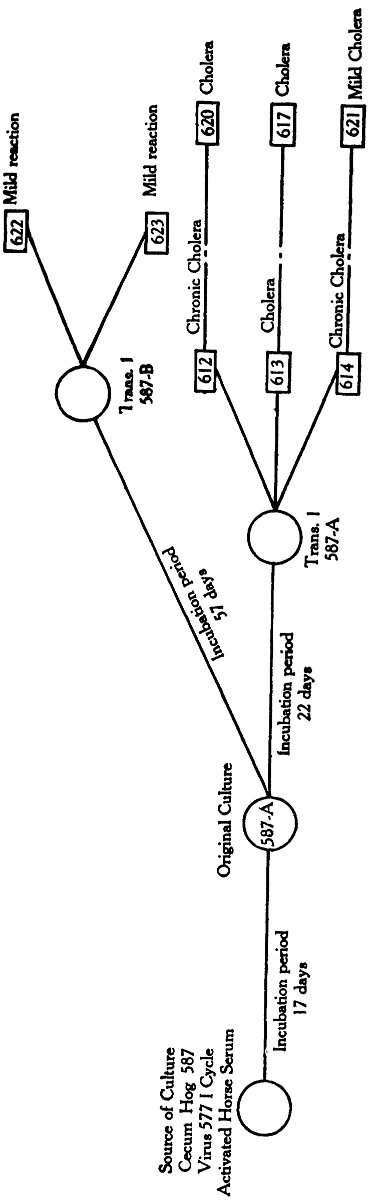
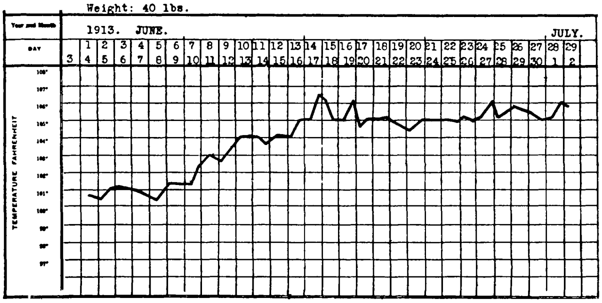


CHART 2.—Graphic representation of expeirments with Transfer 1-587.

HOG 613.

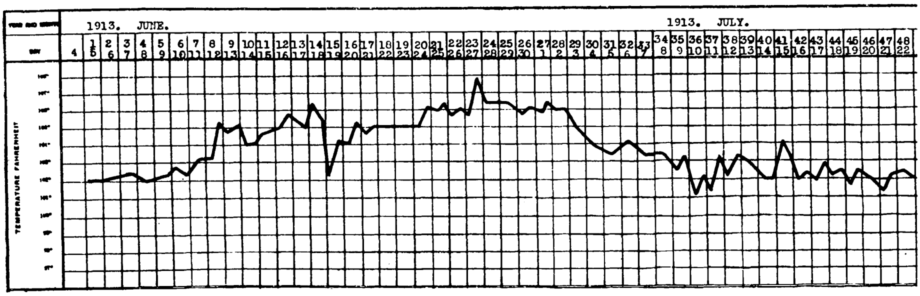


June 3. Intramuscular injection of 1.5 c.c. suspension Culture Transfer 1-587.
June 10. Symptoms appeared.
June 16–July 2. Subacute type of cholera.
July 2. Animal moribund, killed and examined.
Typical lesions.

The animals lost their normal appetite and, during a period of about three weeks following the initial symptoms, became somewhat emaciated. On the fifth week after the inoculation of the animals, rapid convalescence took place and they fully regained normal condition. Both animals had been confined with cholera hogs, but following the experiment they remained healthy and were released as active immunes.

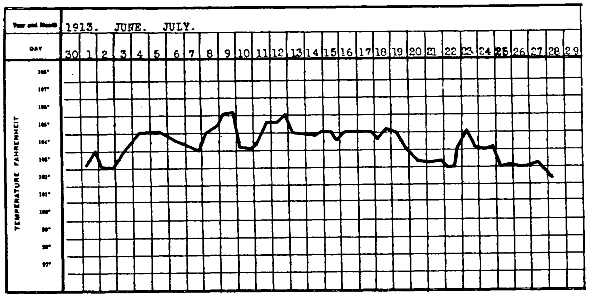
Hog 613 developed a more acute form of the disease than Hogs 612 and 614. After an incubation period of eight days, the temperature rapidly rose and maintained an elevation of approximately 106°–107° for a period of two weeks. During this time Hog 613 manifested the usual symptoms of hog cholera, consisting of anorexia, general weakness and malaise, paralysis of hind quarters, emaciation, and constipation. On July 2, 29 days after inoculation, the animal was killed; on examination, characteristic

HOG 614.



June 4. Intramuscular injection 1.5 c.c. suspension Culture Transfer 1-578.
June 12. Animal "off feed."
June 15–30. Symptoms of chronic type of cholera.
July 4. General improvement noted.
July 15. Animal had been fighting, placed with strange hogs.
July 22. Released as healthy immune.

HOG 620.

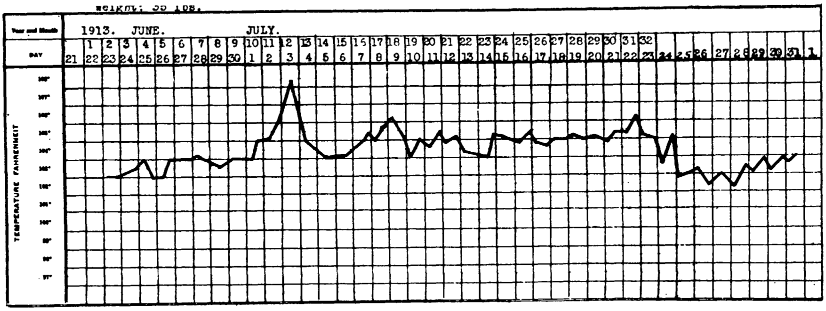


June 30.	Intramuscular injection of 10 c.c. of blood of Hog 612, diluted 1:4, and Berkefeld filtered.
July 4–5.	High temperature—may have been due to excessively hot weather.
July 8.	Symptoms of cholera.
July 10–20.	Gradual emaciation, constipation, anorexia, and malaise.
July 21.	Gradual return of appetite and slow convalescence.
July 29.	Apparently recovered.
July 30.	Animal released as normal.

lesions of hog cholera were found. The heart was normal, the lungs were congested and contained areas of hepatization. The spleen was enlarged, soft, and friable, and engorged with blood. The kidneys were petechiated. The mesenteric and lymphatic glands were enlarged and hemorrhagic, and the congested mucosa of the cecum contained small ulcerated patches near the ileocecal valve. *Spirochaeta suis* was present in the ulcers.

This experiment was controlled in the manner explained in the description of the inoculation of Hog 559 with original culture. Normal hogs were kept in the stable under the same conditions as were these animals. Hogs 612 and 614, which survived

HOG 617.



June 21.	Intramuscular injection of 12 c.c. of blood of Hog 613, diluted 1:4, and Berkefeld filtered.
June 27.	Symptoms appeared.
July 1–15.	Animal very weak, emaciated, constipated, anorexia, and malaise.
July 20.	Some improvement noted.

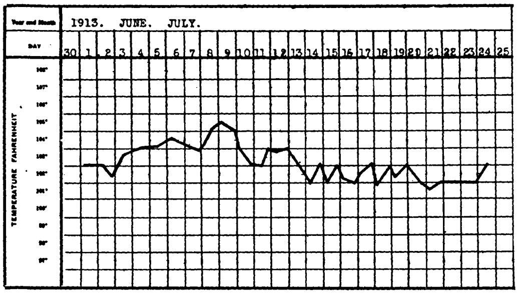
the experiment, possessed active immunity, as was demonstrated by the fact that they were placed with hogs sick with cholera two weeks before being released.

During the period of infection, a sample of blood was obtained from the tail of each of Hogs 612, 613, and 614, used in the experiment above in testing the pathogenicity of Transfer 1-587-A. These samples were defibrinated, diluted 1:4 in sterile water, and passed through the Berkefeld filter. Ten cubic centimeters of the diluted, Berkefeld-filtered blood from Hogs 612, 613, and 614 were injected intramuscularly into Hogs 620, 617, and 621, respectively.

Hog 620, which received the Berkefeld-filtered blood serum from Hog 612, developed symptoms of cholera eight days after inoculation. This case assumed the chronic type extending over two weeks' time after which the animal gradually became convalescent.

Hog 617, which received the Berkefeld-filtered blood from Hog 613 (the animal which developed the most acute form of the disease of the three cases described above), showed unmistakable signs of hog cholera about six days after inoculation. The course of the disease in this animal was of the chronic type and death occurred two months after inoculation.

HOG 621.



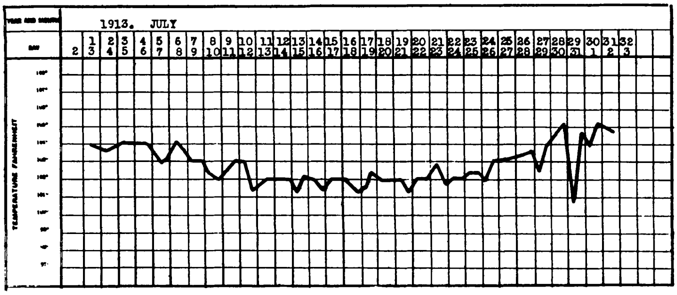
June 30.	Intramuscular injection of 10 c.c. of blood of Hog 614, diluted 1:4, and Berkefeld filtered.
July 6–9.	Animal not normal, appetite poor, general malaise.
July 10.	Condition improved.
July 14.	Condition normal.
July 25.	Released as healthy immune.

Hog 621, inoculated with the Berkefeld-filtered serum of Hog 614, showed mild symptoms of cholera eight days after inoculation. After being slightly "off feed" for two or three days the animal regained its normal condition.

After the conclusion of this experiment, Hogs 620 and 621 were confined for several weeks in an infected inclosure with cholera pigs. No evidences of disease developed, thus establishing the fact that the animals possessed active immunity.

On July 1 one of the transfers, which was made from Strain 587 and which had been kept in the incubator since the time of inoculation, May 5, was submitted to dark field examination. It was found that the culture contained very few organisms which could be recognized as spirochetes, but many granules were present and the small amount of contamination consisted of a micrococcus. This culture, which had been incubated for a period of 57 days, was designated "Transfer 1-587-B," and two hogs,

HOG 622.



July 2. Intramuscular injection of 10 c.c. unfiltered suspension of positive culture No. 587 (May 5, 1913)—(July 2, 1913).

July 7-8. Appetite poor, general malaise, feces normal.

July 9. Apparently normal.

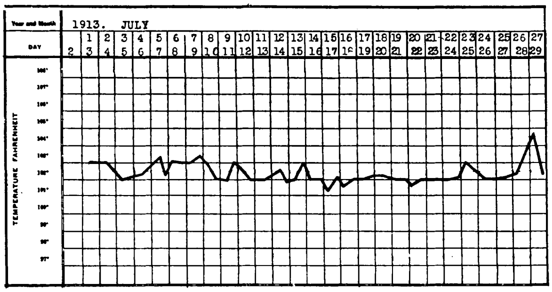
July 22. Exposed to hog cholera; placed with sick animals and injected intramuscularly with 2 c.c. of virus from Hog 631.

July 29–August 2. Symptoms of paralysis and chorea. Appetite fairly good. Animal not emaciated.

August 3. Animal found dead and examined.

Typical lesions of hog cholera absent; intestinal mucosa normal. Culture made from edematous fluid of muscular tissue shows *Str. pyogenes* present.

HOG 623.



July 2. Intramuscular injection of 10 c.c. unfiltered suspension of positive culture No. 587 (May 5, 1913)—(July 2, 1913).

July 7-8. Appetite poor, general malaise, feces normal.

July 9. Apparently normal.

July 22. Exposed to hog cholera; placed with sick animals and injected intramuscularly with 2 c.c. of virus from Hog 631.

July 29. Animal showed symptoms of paralysis and chorea. Could not walk.

July 30. Found dead and examined.

Mucous membrane of small and large intestine congested. No ulcers present in cecum. Typical lesions of cholera absent.

Nos. 622 and 623, were each injected intramuscularly with 10 c.c. of the suspension of this culture in sterile water. Six days after the inoculation of these animals, they showed, in addition to slight temperature changes, mild symptoms of illness. The appetite was poor and the animals were inclined to be listless and inactive. After about two days of inactivity and loss of appetite, they again resumed a normal condition and continued so until exposed to cholera.

On July 22 these animals were exposed to the disease by inoculation, natural exposure, and access to carcass of hog dead from cholera. On July 28 both hogs, Nos. 622 and 623, developed symptoms of paralysis and chorea. On July 29 Hog 623 was found dead and autopsy did not show lesions of hog cholera. Dark field examination of the mucosa of the cecum failed to show any spirochetes. Hog 622, on July 30, showed a moderate rise in temperature, but did not lose appetite or manifest other symptoms of cholera. The right front leg became enormously swollen. On August 3 Hog 622 was found dead. No characteristic lesions of hog cholera were present and cultures made from the serous fluid in the swollen leg developed *Str. pyogenes*. Several preparations were made from the mucosa of the cecum and were submitted to dark field examination with negative results.

On account of the absence of typical symptoms and lesions of hog cholera, also the course of the disease in the control animal (see notes below on Hog 641) and the presence of *Str. pyogenes* in the edematous tissue of Hog 622, it seemed probable that Hogs 622 and 623 died from infection other than hog cholera.

III. TRANSFER 2. THIRD GENERATION ON ARTIFICIAL MEDIA.

On May 27 a number of transfers were made from Culture Transfer 1-587-A. These cultures were incubated under anaerobic conditions from May 27 to July 2, when they were removed from the incubator and examined by the dark field method. One of these transfers, designated "Transfer 2-587-A," contained very little contamination represented by one species of micrococcus and, while only a few spirochetes could be found scattered through the medium, the culture contained many granules. This culture, Transfer 2-587-A, was macerated in sterile water, and on July 3, Hogs 627 and 628 were each inoculated, intramuscularly, with 10 c.c. of the suspension of this transfer. Five days after inoculation both of these animals showed indications of indisposition. From July 8 to July 12, the appetite was poor and the animals were inactive. On July 14 the condition of both hogs was much improved and on July 16 both were pronounced apparently healthy.

On July 22 these animals were exposed to hog cholera in the same manner as Hogs 622 and 623, described above. After seven days' incubation both animals developed symptoms of cholera. Hog 627 succumbed to the disease 22 days after exposure. Hog 628, after suffering from the disease in chronic form, gradually became convalescent and on September 1 was released. Both animals showed greater resistance and maintained a better clinical condition than did the control Hog 641.

On July 11, during the period in which Hog 628 was showing a general reaction from the injection of Transfer 2-587-A, a few cubic centimeters of blood were drawn from the tail, defibrinated, diluted 1:4 in sterile water, and filtered through a bacteria-proof Berkefeld. On July 15 Hog 635 received intramuscularly 5 c.c. of the Berkefeld-filtered serum, diluted 1:4, of Hog 628. This animal showed mild symptoms five days after inoculation and soon developed hog cholera of the subacute type. Four weeks after inoculation Hog 635 was found dead. Autopsy revealed typical lesions.

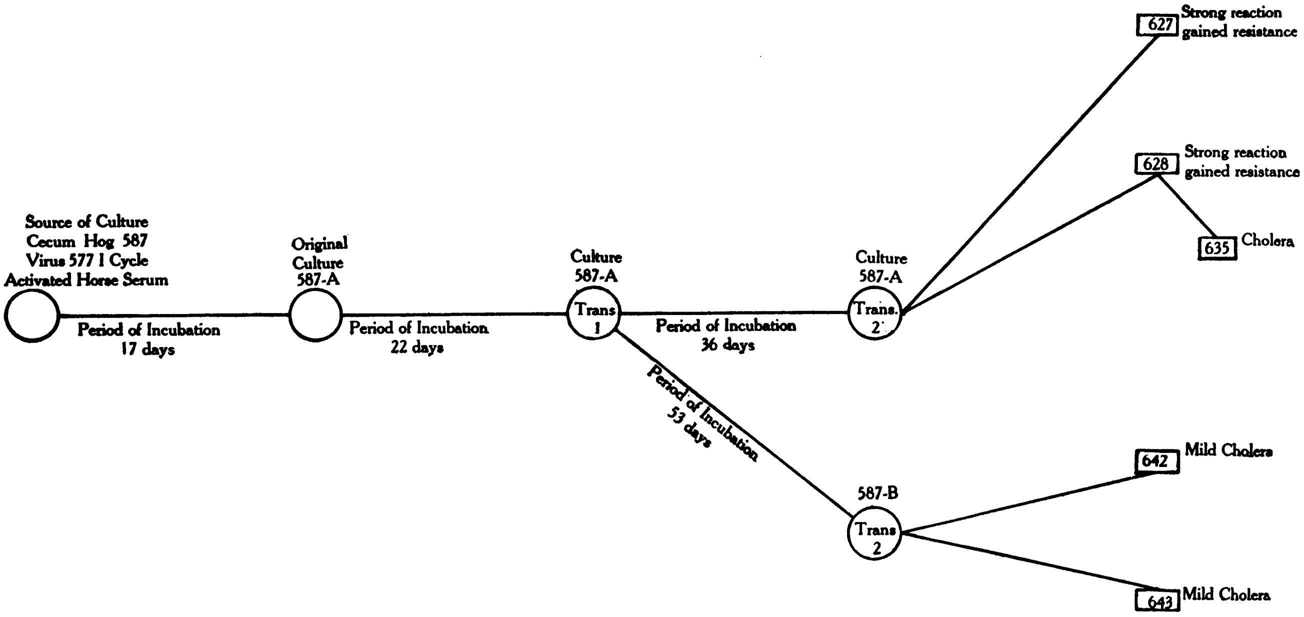
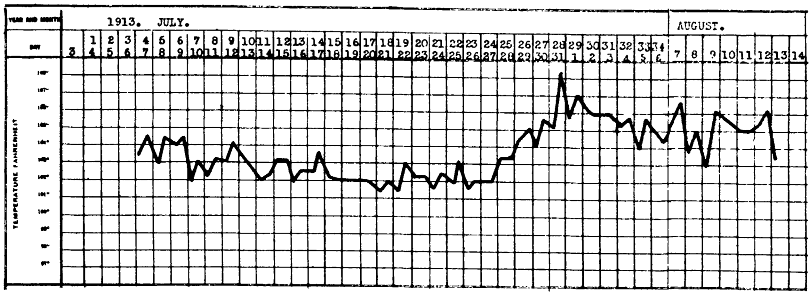


CHART 3.—Graphic representation of experiments with Transfer 2–587.

On July 24 Hogs 642 and 643 each received intramuscularly 5 c.c. of suspension in physiologic salt solution of Culture Transfer 2-587-B. Eight days after inoculation both animals manifested symptoms of mild cholera. They continued to show inactivity, anorexia, arched back, mild blepharitis, and roughened coat for a period of five days, after which they apparently regained a normal condition, which was followed by a recurrence in the case of both Hogs 642 and 643, in seven and twelve days, respectively. Both cases terminated fatally.

HOG 627.



July 3.	Intramuscular injection of 10 c.c. unfiltered suspension culture No. 587 (May 27, 1913)—(July 3, 1913).
July 8–12.	Poor appetite, inactive, eyes dull.
July 12.	Nervous symptoms.
July 14.	Condition improved.
July 16.	Animal apparently normal.
July 22.	Intramuscular injection 2 c.c. Virus Strain 631.
July 23.	Had been fighting with strange hogs.
July 29.	Animal suffering from a relapse, increasing emaciation; symptoms of acute type of cholera.
August 13.	Animal dead.

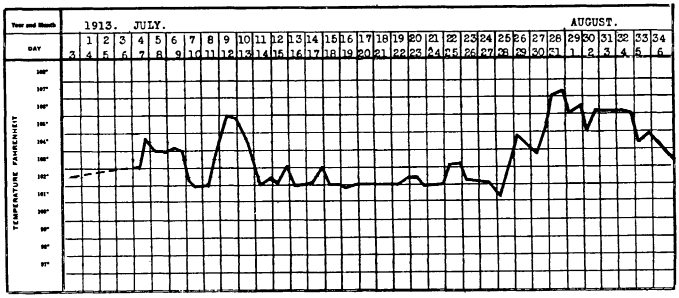
Typical lesions of hog cholera, subcutaneous tissue, lungs, kidney, lymphatic glands, cecum.

IV. FILTERED CULTURES.

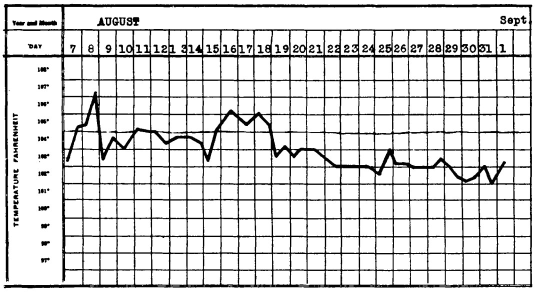
a) *Transfer 1.*—Transfer 1-587-B, described above, a suspension of which was inoculated into Hogs 622 and 623, was also utilized in filtration experiments, which were first suggested by our colleague, Dr. N. S. Ferry. This culture, which had been incubated for a period of 57 days, after being macerated in sterile water in the proportion of one part culture to ten parts water, and thoroughly shaken for about one hour, was passed through a Berkefeld filter. The filter used was a bacteria-proof Berkefeld filter, pressure less than one atmosphere, time of filtration about 20 minutes, and volume of filtrate about 15 c.c. Cultures from this filtrate were made, both aerobically and anaerobically, and no bacterial growth resulted. Dark field examination of the filtrate revealed only relatively few granules. Hogs 624 and 625, on July 3, each received intramuscularly 10 c.c. of filtered culture, Transfer 1-587-B, diluted 1:10 in sterile water. Five days after inoculation both of these animals gave evidences of illness. From July 8 to July 10, the appetite was poor, eyes dull, and animals listless. On July 11 the condition of both hogs was improved and on July 16 they were apparently healthy.

These hogs, Nos. 624 and 625, were exposed with control Hog 641 (see p. 482), by inoculation and by natural exposure. Three days after exposure Hog 624 showed evidences of illness, apparently due to gastritis and autointoxication, resulting from the ingestion of pus from an infected carcass. After a few days this animal fully recovered and exhibited a thrifty condition throughout the experiment, thus proving the existence of active immunity. Hog 625 showed scarcely any reaction as the result of the exposure, and was considered as being actively immunized by the filtered Culture Transfer 1-587-B.

HOG 628.



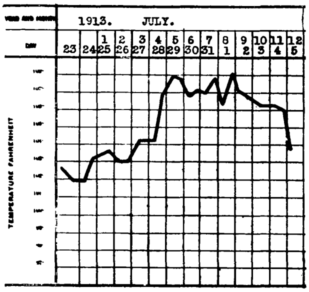
HOG 628 (*continued*).



July 3.	Intramuscular injection of 10 c.c. unfiltered suspension culture No. 587 (May 27, 1913)—(July 3, 1913).
July 8–13.	Inactive, anorexia.
July 15.	Animal apparently normal.
July 22.	Intramuscular injection 2 c.c. Virus Strain 631.
July 30–August 5.	Symptoms of chronic type of cholera.
August 6.	Emaciated and growing progressively weaker.
September 1.	Animal normal, recovered and released.

On July 9, during the reaction following inoculation with filtered culture, a specimen of blood taken from the tail of Hog 625 was defibrinated, diluted 1:4 in sterile water, and filtered through the Berkefeld filter. On July 15, Hog 634 received intramuscularly 5 c.c. of the diluted, filtered blood from Hog 625. Five days after inoculation of Hog 634 the temperature rose two degrees, the animal was "off feed" and

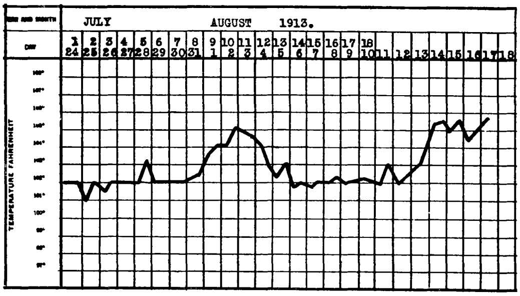
HOG 641.



July 24. Intramuscular injection of 2 c.c. virus.
July 29. Typical symptoms of hog cholera.
August 5. Animal died.

Lesions: lungs, areas of hepatization; spleen, enlarged, soft and friable, engorged with blood; kidneys, petechiated; lymphatic and inguinal glands, enlarged and hemorrhagic; cecum, mucosa congested, small ulcers near ileocecal valve. *Spirochaeta suis* present.

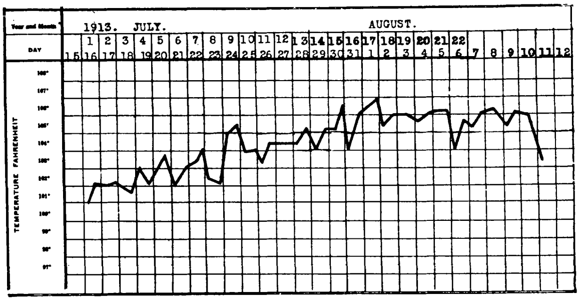
HOG 642.



July 24. Intramuscular injection 5 c.c. physiologic salt suspension Culture 587 (May 27, 1913)—(July 20, 1913), Berkefeld filtered.
August 1. Symptoms of anorexia; malaise, slight blepharitis.
August 5. Animal normal.
August 10. Temperature normal, tho the appearance was not normal.
August 14. Indication of relapse; typical symptoms of cholera.
August 18. Animal found dead.

Typical lesions of hog cholera; subcutaneous tissue, heart, lungs, spleen, kidney, lymphatic system, cecum; *Spirochaeta suis* present.

HOG 635.



July 15. Intramuscular injection of 5 c.c. blood serum of Hog 628, diluted 1:4, and Berkefeld filtered.
July 20–22. Animal "off feed," dull, inactive.
July 24. Condition improved; appetite poor.
July 29–August 5. Symptoms of subacute type of cholera.
August 6. Nearly moribund.
August 11. Animal died.

Typical lesions of hog cholera: lungs, heart, spleen, kidney, cecum, and lymph glands.

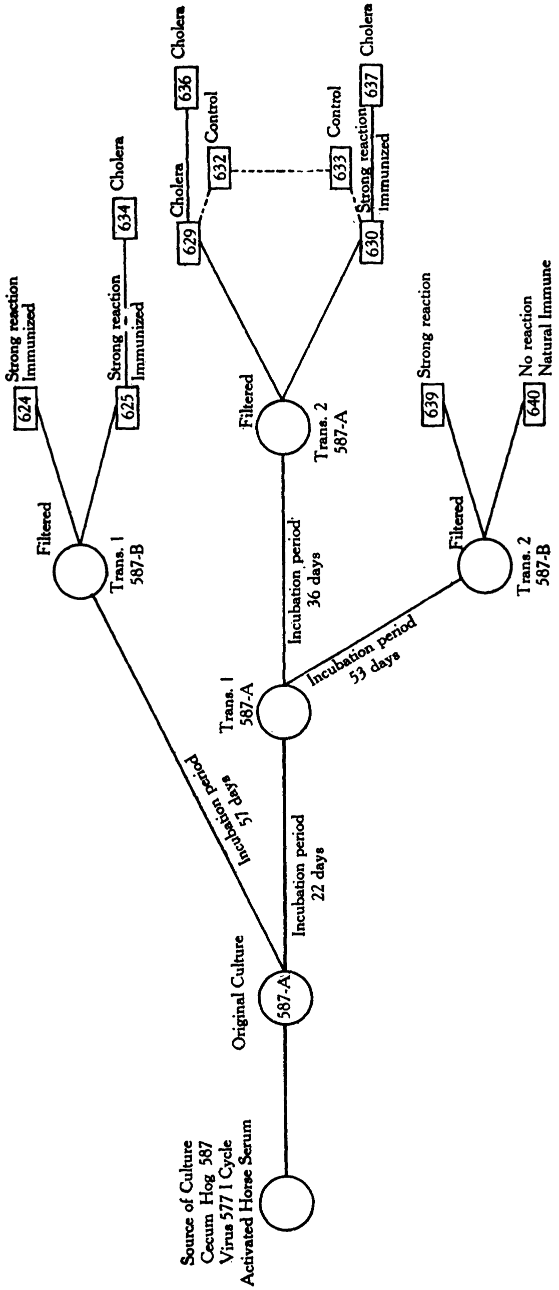
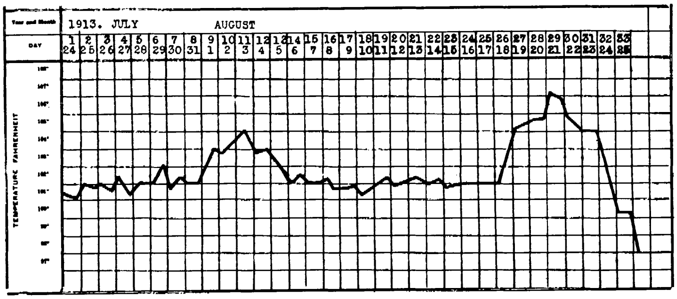


CHART 4.—Graphic representation of experiments with filtered cultures.

HOG 643.



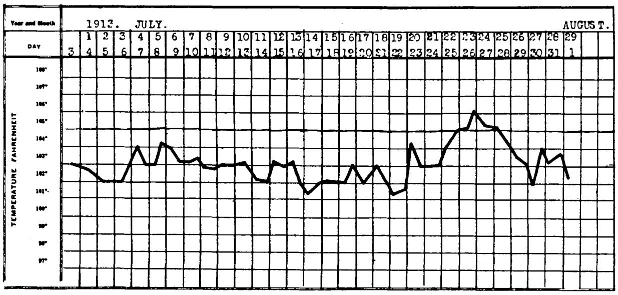
July 24. Intramuscular injection 5 c.c. physiologic salt suspension Culture 587 (May 27, 1913)—(July 20, 1913), Berkefeld filtered.
August 1. Symptoms of anorexia, malaise, slight blepharitis.
August 5. Animal normal.
August 10–17. Animal apparently normal.
August 23. Typical case of hog cholera.
August 26. Animal found dead.

Typical lesions of hog cholera: heart, lungs, spleen, kidney, lymphatic glands, subcutaneous tissue, and cecum.

became constipated. This animal developed cholera of the subacute type and was found dead about four weeks after inoculation.

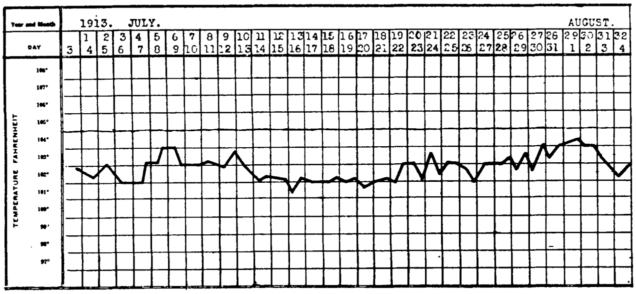
b) *Transfer 2.*—Culture Transfer 2-587-A, described above, on July 2 was filtered and treated in exactly the same manner as described concerning the Berkefeld filtration of Culture Transfer 1-587-B. Transfer 2-587-A had been grown in the incubator for 36 days. On July 3 Hogs 629 and 630 were each injected intramuscularly with

HOG 624.



July 3. Intramuscular injection of 10 c.c. Culture 587 (May 5, 1913)—(July 2, 1913), diluted 1:10, and Berkefeld filtered.
July 8–9. Anorexia, dull, listless.
July 10. Condition improved.
July 13. Animal apparently normal.
July 22. Intramuscular injection 2 c.c. Virus Strain 631.
July 23. Animal had been fighting with strange hogs.
August 1. Animal normal. Released.

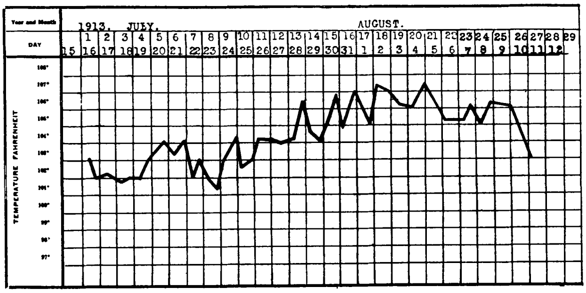
HOG 625.



July 3. Intramuscular injection of 10 c.c. Culture 587 (May 5, 1913)-(July 2, 1913), diluted 1:10, and Berkefeld filtered.
July 8-9. Poor appetite, eyes dull, inactive.
July 14. Animal apparently normal.
July 22. Intramuscular injection 2 c.c. Virus Strain 631.
August 4. Animal released.

10 c.c. of Culture Transfer 2-587-A, diluted 1:10, and filtered through bacteria-proof Berkefeld. About eight days after inoculation, both animals showed unmistakable symptoms of hog cholera. Hog 630, however, exhibited a milder case than Hog 629. Eleven days after inoculation Hog 630 quickly regained normal condition, rise in temperature subsided by crisis, and on July 17 the animal was pronounced normal. Subsequent exposure with control Hog 641 (see p. 482) demonstrated a condition of active immunity. Hog 629 developed a subacute case of hog cholera. The course of this disease lasted for weeks. This animal manifested typical symptoms of the disease and after July 12 the more important symptoms noted were anorexia, malaise,

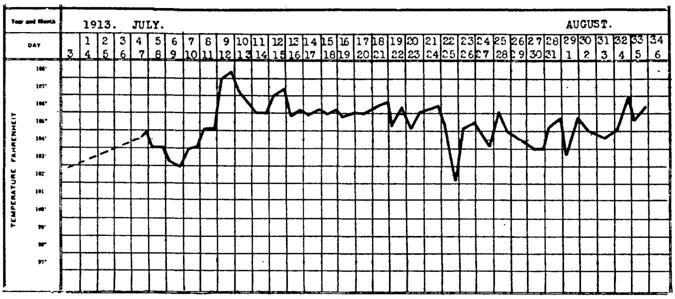
HOG 634.



July 15. Intramuscular injection of 5 c.c. blood serum of Hog 625, diluted 1:4, and Berkefeld filtered.
July 20-21. Inactive, poor appetite, somewhat constipated.
July 24. Apparently normal.
July 27-August 5. Symptoms of subacute type of cholera.
August 6. Nearly moribund.
August 12. Animal found dead.

Typical lesions of hog cholera in subcutaneous tissue; heart, lungs, kidney, spleen, cecum, and lymph glands.

HOG 629.



July 3. Intramuscular injection 10 c.c. filtered Culture 587 (May 27, 1913)—(July 3, 1913), 1:10, Berkefeld filtered.

July 9. Appetite poor.

July 10–20. Symptoms of subacute type of cholera: anorexia, blepharitis, malaise, roughened coat, emaciation, and profuse diarrhea.

July 24. Improvement noted.

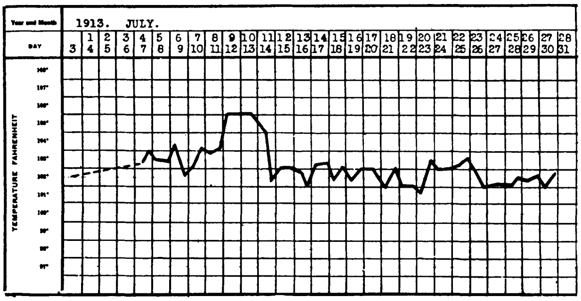
July 28. Relapse.

August 6. Nearly moribund.

August 7. Animal found dead.

Lesions: heart, ecchymosis on auricles; lungs, complete consolidation; spleen, enlarged, soft, friable; hemorrhagic; kidney, few petechiae; cecum, ulcers near ileocecal valve; inguinal and other lymph glands, enlarged, hemorrhagic; adhesions of ascending colon to peritoneum; extravasation in subcutaneous tissue. *Spirochaeta suis* present.

HOG 630.



July 3. Intramuscular injection of 10 c.c. filtered Culture 587 (May 27, 1913)—(July 3, 1913), 1:10, Berkefeld filtered.

July 8. Poor appetite.

July 9–14. General inactivity, poor appetite, no apparent emaciation or loss of general tone.

July 15. Appetite good.

July 17. Apparently normal.

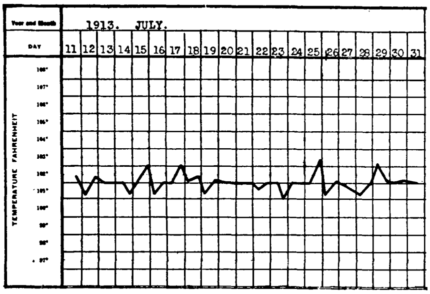
July 31. Animal normal. Released.

blepharitis, emaciation, and roughened coat. Hog 629 was found dead on August 7, 35 days after inoculation.

As a control on this experiment, Hogs 629 and 630, on July 11, were transferred from the room which they had occupied in the experimental stable to another, and, after disinfection of the room, normal Hogs 632 and 633 were immediately placed in the quarters formerly occupied by sick Hogs 629 and 630. An examination of the dates on the clinical charts will show that this rearrangement was made during the height of the disease in Hogs 629 and 630. Hogs 632 and 633 were subjected to exactly the same conditions as those attending the care and treatment of Hogs 629 and 630 except, of course, that they received no inoculation with infectious material. These control animals remained perfectly normal in every way and after being kept as controls for some time were utilized in experiments.

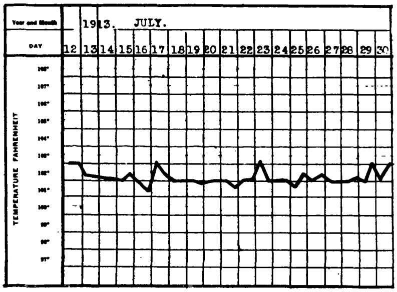
Hogs 636 and 637 on July 15 each received an intramuscular injection of 5 c.c. of the defibrinated, diluted, and Berkefeld-filtered blood serum of Hogs 629 and 630, respectively. Both of these animals developed typical symptoms of hog cholera and both were moribund 26 days after inoculation. Practically the only difference observed in their clinical appearance was that Hog 636, which had received the filtered blood from Hog 629, showed more severe symptoms of the disease after a shorter incubation period than those manifested in Hog 637, which had been inoculated with the blood from Hog 630. This result was consistent, as the injection of the filtered culture described above into Hog 629 produced the subacute type of hog cholera, while Hog 630, after a marked reaction, quickly regained normal condition.

HOG 632.



Normal hog.

HOG 633.



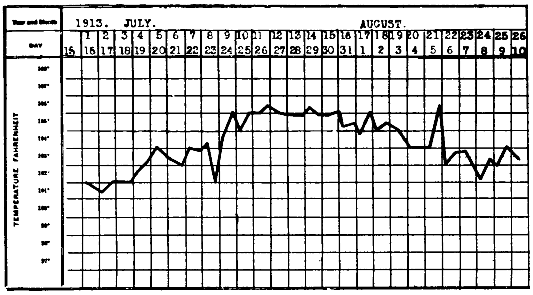
Normal hog.

In order to make the present experiment more complete Hogs 639 and 640 were injected each intramuscularly with 4 c.c. of suspension of Culture Transfer 2-587-B, which, after 53 days' incubation, had been macerated in sterile water, diluted 1:6, and subjected to filtration through the Berkefeld.

From the fifth to the ninth day following inoculation, Hog 639 showed a marked reaction. During this period the animal was somewhat inactive and had poor appetite. Nine days after inoculation Hog 639 regained normal condition.

Hog 640 manifested no apparent reaction from the inoculation. This was regarded as an indication of natural immunity, as Hog 640 is a large brood sow and is probably a "natural immune."

HOG 636.



July 15. Intramuscular injection of 5 c.c. blood serum of Hog 629, diluted 1:4, and Berkefeld filtered.

July 21. Animal "off feed" and constipated.

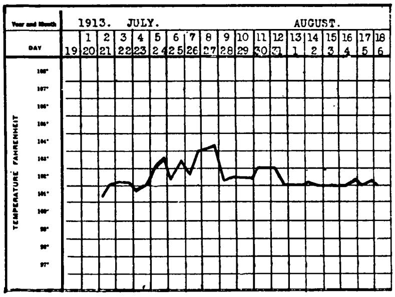
July 22–August 5. Typical symptoms of subacute type of cholera.

August 6. Animal nearly moribund.

August 11. Animal killed.

Typical lesions of hog cholera in lungs, spleen, kidney, cecum, and lymph glands.

HOG 639.

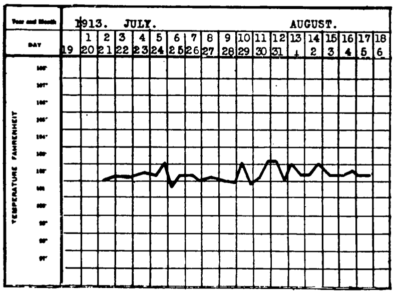


July 19. Intramuscular injection of 4 c.c. suspension Culture 587 (May 27, 1913), dilute 1:6, and Berkefeld filtered.

July 24–28. Anorexia, inactivity.

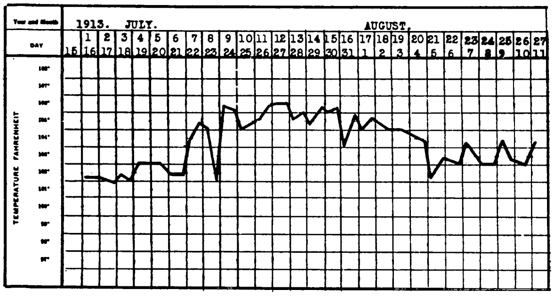
July 29. Normal.

HOG 640.



July 19. Intramuscular injection of 4 c.c. suspension, Culture 587 (May 27, 1913), diluted 1:6, and Berkefeld filtered.

HOG 637.



July 15. Intramuscular injection of 5 c.c. blood serum of Hog 630, diluted 1:4, and Berkefeld filtered.

July 21. Appetite poor; constipated.

July 22. Anorexia, constipation and diarrhea, weakened condition, emaciation.

August 6. Animal nearly moribund.

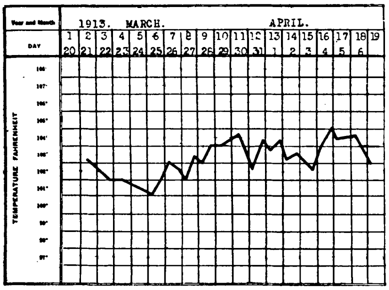
August 12. Animal killed.

Typical lesions of hog cholera; lungs, kidney, lymph glands, mucosa of cecum congested.

V. UNSUCCESSFUL INOCULATIONS WITH CULTURES, CONTROL ON POSITIVE RESULTS.

a) *Strain 576.*—Hog 576, on March 20, was inoculated intramuscularly with 5 c.c. of hog cholera virus, strain Kansas III. This animal developed typical symptoms of cholera and, being moribund 18 days after inoculation, was bled and examined. The ordinary lesions of hog cholera were present and the material from the ulcers in the cecum, when examined by the dark field method, showed numerous spirochetes and spirilla of various forms. From this material in the ulcers, cultures were made, and after three days' incubation some of these cultures were submitted to dark field examination. On account of the fact that some large forms of spirochetes were present, which at that time were regarded as possibly the same form which had repeatedly been found in the blood of cholera hogs, Culture 576-A was macerated in physiologic salt solution and 5 c.c. inoculated intravenously into Hog 584.

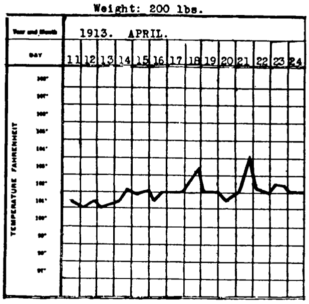
HOG 576.



March 20. Intramuscular injection of 5 c.c. Kansas III virus.
March 29. Animal not eating, general symptoms appearing.
April 4. Animal weak.
April 8. Moribund, bled from the carotid artery.

Typical lesions: lungs, heart, spleen, lymph glands; ulcers in cecum.

HOG 584.



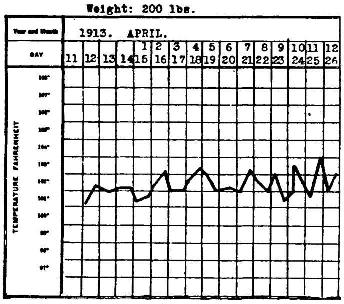
April 11. Intravenous injection of 5 c.c. physiologic salt suspension, Culture 576.

Hog 584, which received Culture 576-A, showed a slight thermal reaction on April 18, seven days after injection with the culture. Again on April 21, the temperature rose two degrees. These thermal reactions were possibly due to the presence of a variety of contaminating organisms in the suspension, as no clinical symptoms were apparent in the animal. On April 25 the animal was released as normal and subsequent inoculation with hog cholera serum showed that Hog 584 was susceptible to the disease.

From the original culture, 576-A, transfers were made on April 11 and incubated under anaerobic conditions. Transfer 1-576-A, six days after inoculation, on being submitted to dark field examination, showed the presence of relatively large spirochetes, similar to those which had been found in the original culture, 576-A. Therefore, Transfer 1-576-A was suspended in physiologic salt solution and 2.5 c.c. of the suspension injected intravenously into Hog 585, on April 14. Five days after inoculation the animal showed an irregular temperature ranging between 102° and 103°, and this variation in thermal condition was present for several days following. However, no symptoms of hog cholera were apparent and it was concluded that the fluctuating temperature was possibly due to the injection of contaminating organisms which were present in Transfer 1-576-A. The animal was finally released, and on being injected later with horse serum virus, hog cholera was produced and death eventually resulted.

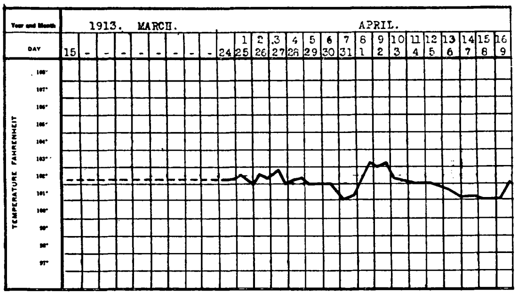
b) *Strain 560.*—On February 14 Hog 560 received 5 c.c. of Kansas III virus intramuscularly. Six days after inoculation the animal developed symptoms of acute cholera and after a duration of the disease for five days, being moribund, was bled and examined. Typical lesions of cholera were found on autopsy. Numerous spirochetes of various forms were found in the necrotic material contained in the ulcers of the cecum. Inoculations into culture media were made in the usual way and after five days' incubation one of these cultures, designated as "Culture 560-A," after being examined on the dark field, was found to contain a growth of spirochetes. Contamination with various forms of bacteria and cocci, however, was present. In an attempt to eliminate as much of this contamination as possible, Culture 560-A was transferred into various culture media containing different chemicals, for the purpose of exerting slight germicidal action and the possible inhibition of the extraneous organisms. On March 24, after 21 days' incubation, Transfer 1-560-A, which had been made in a horse serum medium containing a small percentage of formol, was found, on dark field examination, to contain a few spirochetes, but no granules were observed. Two cubic centimeters of a physiologic salt suspension of Transfer 1-560-A were inoculated intravenously, March 24, into Hog 571. This animal showed no apparent result following the inoculation of this culture, except for a slight reaction which occurred during the eighth and ninth days after the inoculation. Subsequent injection of Hog 571 with horse serum virus resulted in the death of the animal from hog cholera of the acute type.

HOG 585.



April 14.	Intravenous injection of 2.5 c.c. physiological salt suspension, Transfer 1, Culture 576.

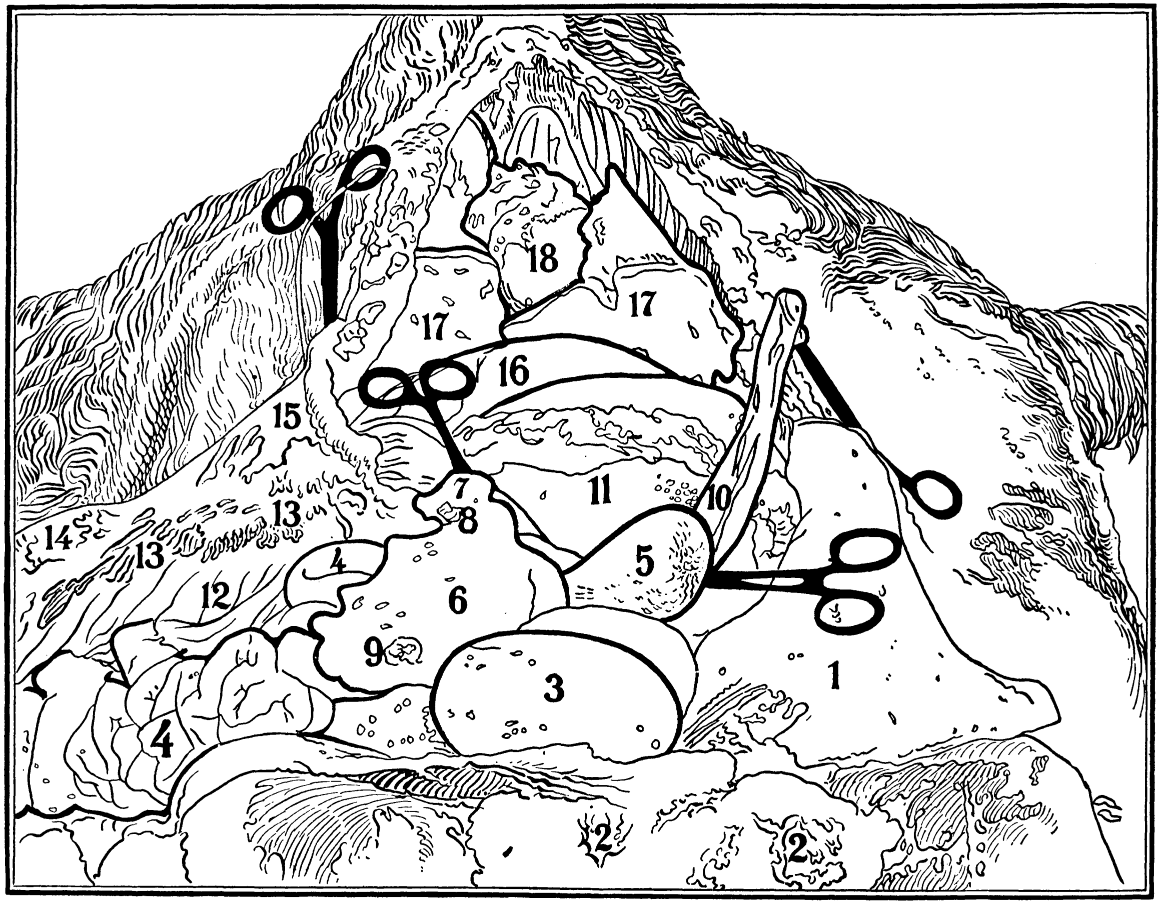
HOG 571.



March 15.	Blood normal.
March 15–24.	Animal normal.
March 24.	Intravenous injection of 2 c.c. physiological salt suspension, Culture 1, Transfer 2, Formol (March 3, 1913), Hog 560.
March 31.	Animal normal.
April 3.	Animal a little "off feed."

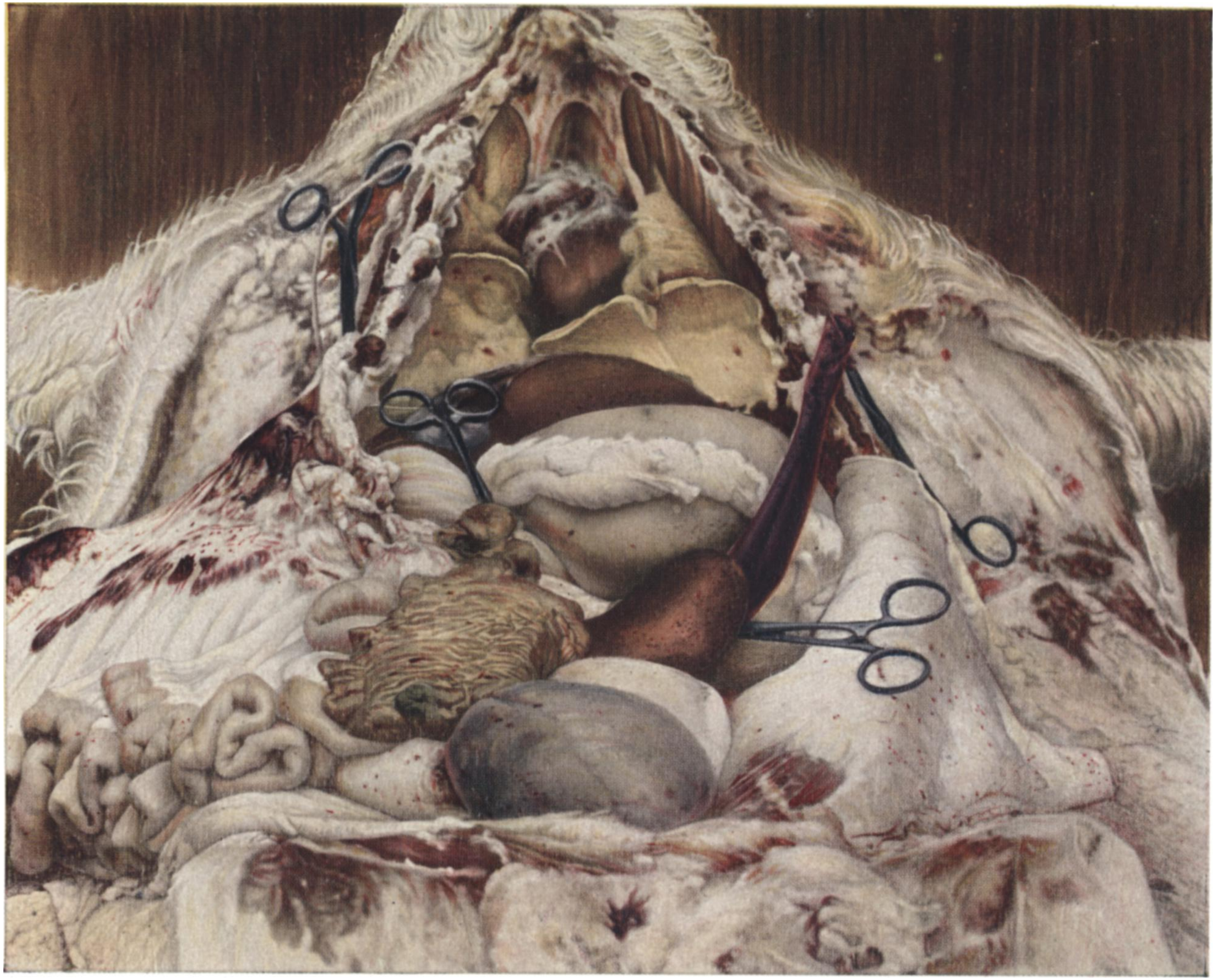
This includes all of those experiments which proved unsuccessful, and serve as admirable controls on the results given. It will be noticed that the unsuccessful experiments included under the last heading were conducted during the months of March and April. At that time it had not been definitely determined that the crypts of the ceca of normal hogs may contain spirochetes and spirilla. In so far as our observations go, these organisms are usually relatively large and altogether unlikely to be related to the form which we have designated as *Spirochaeta suis*, the latter being found in the intestinal ulcers and the external local lesions of cholera hogs. No doubt, the small spirochete described in the first part of this paper as *Spirochaeta suis* was present in the ulcers of Hogs 576 and 560, this material being used in the inoculation of the cultures described under the present heading. However, all of the inoculations into hogs, of cultures from these two strains, were made after a comparatively short period of incubation. This explains the failure to produce positive symptoms of hog cholera with the cultures, as our later results reported above indicate that it is necessary to incubate the cultures for several weeks in order to grow *Spirochaetae suis*.

The most important bearing, however, of these unsuccessful attempts to produce the disease with the cultures noted under the last heading pertains to the control which they give on the results described in the preceding pages. The foregoing positive results from inoculations of filtered and unfiltered cultures of *Spirochaeta suis* are lacking in one detail. Without some means of controlling the results, it might be logically assumed that those animals which had received hog cholera from the injection of cultures and transfers did so as the result of the injection of a small amount of original virus, which possibly might have been transferred through the cultures. This we know to be untrue because of the failure to produce hog cholera in animals inoculated with cultures and transfers from Strains 576 and 560. Positive results followed the inoculation of Hog 559 with the original culture, 551-A, which had an incubation period of only five days. The result may have been due to some of the original virus having been transferred to the animal through the culture. However, we believe that the



1, Peritoneum (showing small blood extravasations). 2, 2, Inguinal Glands (enlarged and hemorrhagic). 3, Bladder (showing small blood extravasations). 4, Small Intestine (Ileum) (covered with punctiform hemorrhages). 5, Kidney (petechiated). 6, Cecum (ental surface showing excessively congested mucosa). 7. Ileocecal Valve. 8, 9, Typical button ulcers in cecum. 10, Spleen (enlarged, soft and gorged with blood). 11, Stomach. 12, Mesentery (showing minute hemorrhagic spots). 13, 13, Mesenteric Glands (enlarged and excessively hemorrhagic). 14, 15, Small Intestine (Jejunum) (containing ulcerated mucosa and extensive hemorrhage). 16, Liver. 17, 17, Lungs (showing areas of congestion, hemorrhage, and solidification). 18, Heart (small blood extravasations in muscle).

PLATE 4.



Hog 559.—Inoculated 22 days previously with suspension of culture from cecum of Hog 551.

succeeding results are sufficiently controlled by the facts that Hog 584 failed to develop hog cholera after inoculation with Culture 576-A, and that Hogs 571 and 585 did not respond to the injection of Transfers 1-576-A and 1-560-A, respectively.

CONCLUSIONS.

Spirochaeta suis is an organism found in the intestinal ulcers, crypts in the ceca, and external local lesions of animals suffering from hog cholera. It is a typical spirochete, simulating in many characteristics *Spirochaeta pallida*, *Spirochaeta gallinarum*, and other forms whose morphology and life history are becoming better understood. *Spirochaeta suis* appears to be capable of breaking up into granules and these granules may play an important part in the life cycle and physiological functions of the organism. They are present in the blood of cholera hogs, in cultures of the *Spirochaeta suis*, and are capable of producing the disease in healthy hogs.

In the blood of hogs suffering from cholera the presence of a relatively large spirochete in few numbers has been recognized. As this organism has not been found in the blood of normal hogs it may represent *Spirochaeta suis* in a transitional form. Its morphological variation from *Spirochaeta suis*, as found in ulcers and local foci, may be due to the unfavorable action of blood as a medium or to its natural processes as a part of the life cycle of the species.

Spirochaeta suis is an obligatory anaerobic organism and usually requires several weeks' incubation for growth to take place on artificial culture medium. It may be transferred from generation to generation on artificial culture medium. Cultures containing the organism in the form of granules and spirochetes may be passed through bacteria-proof filters and the spirochetes removed, the few small granules which pass through being capable of producing hog cholera or resistance to the disease.

Spirochaeta suis is capable of producing typical hog cholera when injected into healthy hogs. This is true not alone of contaminated cultures made directly from the intestinal ulcers of cholera hogs; second and third generations on artificial culture media, containing the *Spirochaeta suis*, as well as the Berkefeld filtrates of the same transfers, are capable of producing hog cholera and marked reactions, which confer more or less protection against the disease. The pathogenicity of these cultures does not appear to be due to the passage of an unknown "invisible microorganism" which is finally transmitted to healthy hogs by inoculation. Control experiments tend to show that the pathogenicity of the cultures of *Spirochaeta suis* is due to the species itself in the form of spirochetes or granules.

Finally, in those hogs which receive the disease from cultures of *Spirochaeta suis*, the organism is present in the intestinal lesions or local external lesions, as demonstrated by the dark field examination.

From the above results, which have practically fulfilled Koch's laws, in so far as it is possible with an organism possessing the biological characteristics of spirochetes, it may logically be concluded that *Spirochaeta suis* is more nearly established as the specific cause of hog cholera than any other known organism.